Electrical Determination of Water Content and Concentration Profile in a Simulation Model of In Vivo Stratum Corneum

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Because of its efficient water-holding capacity, healthy stratum corneum (SC) can stay soft and flexible under any environmental conditions. There may be, however, a great difference in water content within the SC between the lowermost layer that faces the wet underlying living tissue and the superficial portion of the SC that is exposed to the relatively dry ambient atmosphere. To better understand the water profile of the SC and also to verify the accuracy of measurements of high frequency conductance used to evaluate the hydration state of the skin surface, we devised a simple and convenient in vitro model of the SC that simulates the in vivo setting of the SC. It consists of an isolated sheet of SC whose lower surface covers a pad of water-saturated filter paper, and its upper surface is exposed to the ambient atmosphere. By placing this model in environments with different relative humidities (RH), we confirmed that the recorded conductance values correlated well with the actual water content of the SC ($r = 0.94$). Using a model having five layers of SC sheets, the water content of the innermost portion of the SC was estimated to be equivalent to about 90% of its dry weight; this level of water content remaining relatively constant over a wide range of ambient RH except in extraordinarily humid environments above 90% RH when water began to accumulate excessively in the whole SC. Using this five SC-sheet model, it was clearly demonstrated that there was an almost straight water concentration gradient from the lowermost layer to the uppermost layer of the SC. We also confirmed that the skin conductance of the high frequency current correlated well with the water content of the superficial portion of the SC as well as with that of the whole SC, therefore, it is a good parameter of the hydration state of the superficial layer of the SC. *J Invest Dermatol 92:854–859, 1989*

The stratum corneum (SC), which is an efficient barrier to water and other substances, plays a crucial role in the protection of humans [1]. In addition, because it is capable of binding a certain amount of water, the SC can stay soft and flexible even in a dry environment, thus allowing free body movement without cracking or scaling of the skin surface. Lack of water from the SC results in a rough, brittle, scaly skin surface. In pathologic scaly skin, it has been shown that the decreased water-holding capacity of the horny layer is closely associated with the impaired water-barrier function [2]. Recent studies have suggested the possibility that if the water-barrier function of this layer is impaired, it may have an effect on the differentiation process of the underlying living epidermal layers [3].

The SC constantly receives water from within the body, and water can also be absorbed from a very humid environment. Because the SC as a whole is a rate-limiting barrier between the fully water-saturated viable tissue and the dry outer environment, through which diffusion of water takes place as a purely passive process, a concentration gradient of water exists within the SC in vivo [4,5].

Recent recognition of the importance of the hydration state of the SC has prompted interest in noninvasive methodology to assess the state of skin surface hydration in vivo. Because of urgent demands for such an approach, a number of techniques have been developed to measure skin properties that are influenced by the hydration state of the horny layer, e.g., assessment based on water evaporation [6] or measurement of electrical [7], spectroscopic [8–10], and mechanical [11,12] properties. Among the various techniques available, the method employing skin conductance of high-frequency current of 3.5 MHz [4] has proven to be useful as a convenient, noninvasive, quantitative technique. The dynamic process of hydration and dehydration of the skin surface can be easily monitored and analyzed with this method by performing a water sorption–desorption test in vivo [13]. This test provides useful information about the altered functions of pathological SC as well as the efficacy of topical moisturizing agents in vivo [14]. It can be used also to evaluate the function of the SC lipids that play an important role in the maintenance of the hydration in SC [15]. When originally reported [4], it was speculated that this method was sensitive to subtle changes in the hydration state of the skin surface because of the skin effect that is observed in a conductor operating at high frequencies. The true depth of penetration of this electric current into the skin, however, was not fully determined. A later preliminary in vitro study [16] demonstrated that a high frequency current might propagate much deeper into the tissue than previously thought.

Until now, all experiments on the hydration state of the SC have used an isolated piece of uniformly hydrated SC. In the present

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Abbreviations:

PBS: phosphate-buffered saline
RH: relative humidity
SC: stratum corneum

study, we devised a simple simulation model of an in vivo SC, in which a concentration gradient of water exists between the surface and the lowermost portion. Using this model we analyzed the hydration state of the SC as well as assessed the accuracy of the high frequency method.

MATERIALS AND METHODS

Sheet of Stratum Corneum Pieces of healthy human skin were obtained from the extensor surface of the amputated thigh of a 58-year-old woman with squamous cell carcinoma. A square sheet of SC about 3 cm in diameter was prepared according to the method described by Kligman and Christophers [17]. First, an epidermal sheet was peeled off the dermis after immersion of the skin piece in hot water (60°C) for 2 min. Then, the SC was separated from the epidermis by enzymatic digestion with 0.0001% trypsin in phosphate-buffered saline (PBS), pH 7.4, for 18 h at 37°C. The resulting holeless SC sheet was rinsed with cold PBS for 2 h and cut square about 2 cm along the edge.

Simulation Model of In Vivo Stratum Corneum We made a simple SC model, as illustrated in Fig 1, which consisted of a sheet of SC that tightly occluded the underlying water-saturated filter paper placed as in a diffusion chamber. The SC sheet was mounted with the skin surface upward on a pad of overlapping 1 cm^2 filter paper saturated with PBS placed on a slide glass, and all the free edges of the SC sheet were sealed to the glass with a removable frame of adhesive vinyl tape and a 6-mm hole in the center (Fig 2). The surface of the SC was exposed to the ambient atmosphere only through the hole, and passage of water was allowed only through this portion of the SC. Therefore, a concentration gradient of water is formed within the SC sheet as noted in vivo [4,5]. The underlying water-saturated filter paper, like fluid-saturated cutaneous tissue in vivo, plays a part as a water source for overlying SC and also as a conducting medium that allows the formation of a sufficient electric field. The number of SC sheets was changed depending on the purpose of the experiments. The terminology used to identify the models employed in this study was based on the number of SC sheets used, e.g., a single-sheet model or a five-sheet model (Fig 1).

Relative Humidity Chamber Environments of different relative humidities (RH), i.e., 33, 69, 75, 90, and 97%, were generated in a sealed chamber by placing various kinds of saturated aqueous salt solutions in a reservoir [18]. An environment of 0% RH was produced in a desiccator containing silica gel.

Conductance Measurement A skin surface hygrometer (IBS Co, Hamamatsu, Japan) was used to measure the conductance by the SC of a high-frequency electric current of 3.5 MHz [4]. In the present study, we used a new sensitive probe consisting of an outer cylindrical electrode, 4 mm in diameter, and a central one, 2 mm in diameter, which gives three times more sensitive measurement than a conventional probe. Measurements were obtained by inserting the probe into each chamber through a small hole in the lid, without disturbing the inner environment.

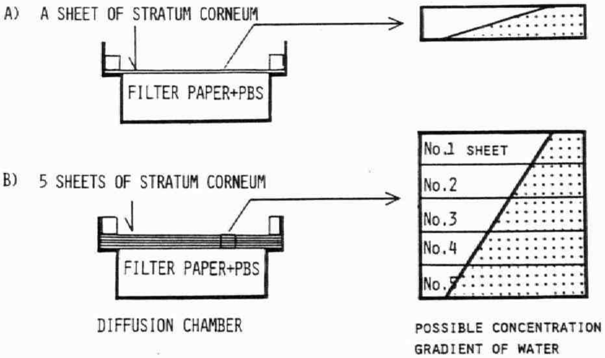

Figure 1. Schematic diagrams of the simulation model with single stratum corneum sheet (single-sheet model) and the simulation model with five sheets of overlying stratum corneum (five-sheet model). Possible water fraction in the stratum corneum is schematically shown by the *dotted* portion.

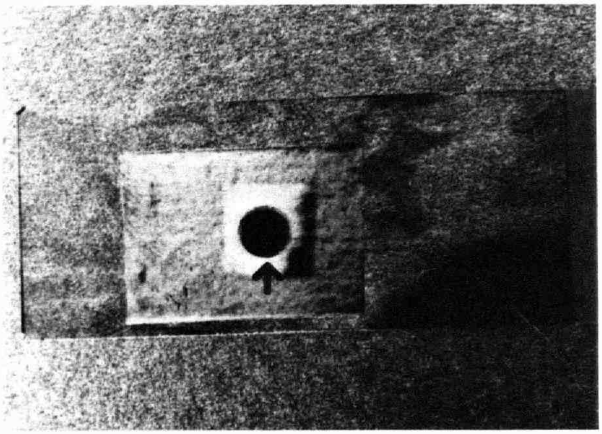

Figure 2. The simulation model of in vivo stratum corneum consisting of a square pad of water-saturated filter paper and an overlying sheet of stratum corneum. They are tightly sealed on a slide glass with adhesive vinyl tape bearing a central round hole *(arrow)* through which water evaporation from the stratum corneum takes place.

Measurement of Water Evaporation Evaporation of water from the surface of the SC was measured using an Evaporimeter, as described by Nilsson [6], for evaluation of transepidermal water loss.

Gravimetrical Determination of Water Content in the Stratum Corneum The dry weight of each SC sheet was measured after 48 h storage in a chamber containing silica gel. Gravimetrical measurements were performed again immediately after conductance measurements. After removal from the model, the SC sheets were weighed as soon as possible to avoid the change of water content. Because the lower aspect of the innermost SC sheet, which directly faced the PBS-saturated filter paper was usually wet, excess PBS was at first blotted with paper before measurement. (The dry SC samples used in the present study were found to contain primary bound water equivalent to 6.93 ± 1.72 mg/100 mg of dry weight of SC; these data obtained by use of Karl Fischer's vaporization method [19] were kindly offered by Mr. H. Kaneko in Pola Lab., Tokyo. This bound water fraction in the SC was not subtracted from the obtained values as in the previous in vitro study [20].)

Experimental Design Three simulation models were used for each experimental condition. They were placed in a chamber for at least 12 h before measurement. The duration of time was long enough to attain equilibrium in the hydration state of SC in each environment as explained below. The change of conductance and the water content of isolated unmounted SC sheets were examined in the same manner.

Conductance values of a piece of the original excised skin sample were also measured and compared with values obtained for the single-sheet model.

RESULTS

Setting of the Model Under usual environmental conditions, conductance values continued to increase gradually after mounting of a dry sheet of SC on the model until the SC reached equilibrium between the environmental air and the water-saturated paper. The recorded values reached a plateau after about 15 min. (In the case of the five-sheet model it took about 60 min.) Water evaporation from the SC mounted in the model was 1 – 3 $g/m^2/h$, slightly lower than the values obtained in vivo. In such a model, if a progressive stripping of the SC with adhesive tape was successfully performed at least to some depth, a gradual increase in conductance as well as water evaporation was clearly demonstrated as deeper layers of the SC were serially exposed (data not shown), in the same fashion as noted in vivo [4].

Thickness of the Underlying Conductive Medium The depth of propagation of the high frequency current was determined by changing the thickness of the underlying water-saturated filter paper. The single SC sheet model with only one underlying sheet of PBS-saturated filter paper was initially kept in an ambient condition with a RH of 45% and a temperature of 26°C for 2 h to obtain an equilibrium of hydration in the SC. Then conductance was measured by increasing the number of new sheets of filter paper up to a total of 40 sheets.

As shown in Figure 3, the value recorded with only one sheet of underlying filter paper was quite low. With an increase in the number of sheets of paper, the conductance value increased until five sheets of paper were in place. At this point the readings reached a plateau with a value around 100 μmho, with no more increase despite further addition of underlying paper. The plateau was close to those measured for pieces of the whole original skin under the same experimental conditions. The total thickness of five sheets of filter paper with PBS was approximately 5 mm. As a result, we decided to use at least five underlying sheets of filter paper for every model in subsequent experiments. Even after keeping these models in an environment of 0% RH for more than 24 h, conductance values did not deviate considerably from the original, and there was no loss in flexibility of the SC to touch, although there was a small decrease of water in the filter paper due to evaporation through the SC.

Correlation Between Water Content in Stratum Corneum and Recorded Conductance Value Figure 4 illustrates the change of water content in the SC in relation to different environmental RH. The water content in an isolated SC sheet, in which water was absorbed only from the environmental air, gradually increased with elevated RH until a dramatic increase occurred at an RH of over 90%. This curve was almost identical to those reported previously [5,16].

In contrast, the SC sheet in the simulation model was much more hydrated, even at 0% RH, because there was a sufficient water supply from the underlying water-saturated filter paper. It should be noted that even under extremely dry ambient conditions of 0% RH, the SC retained, as a whole, 0.446 ± 0.075 mg of water per mg dry SC. After a gradual increase in water content with RH, there was a

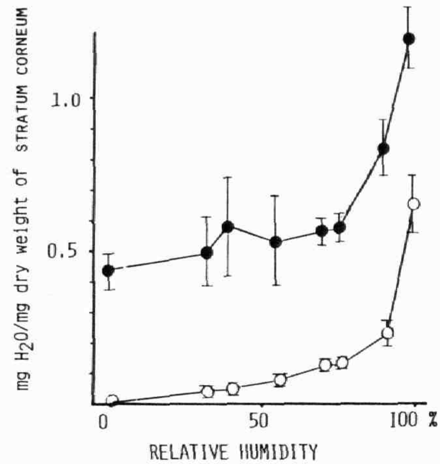

Figure 4. The change of gravimetrically determined water content of the stratum corneum sheet as a function of relative humidity. SC in the single sheet model *(closed circles)*; isolated unmounted SC sheet *(open circles)*.

dramatic increase at higher RH that reached 1.180 ± 0.105 mg water per mg dry SC at 97% RH.

Figure 5 shows the results of parallel recordings of conductance in the same experiment. Although only slightly lower than the conductance values recorded on the whole skin sample the pattern of the conductance curve obtained from the simulation model was quite similar to that of the former and corresponded to that of the water content shown in Figure 4. Conductance was almost unmeasurable on an isolated SC sheet placed in ambient conditions of 0 to 64% RH and a low value of 28.4 ± 6.3 μmho was noted at 97% RH.

The correlation between the water content and the conductance obtained with the single sheet model was very high (Fig 6). The data seemed to fit a straight line with a correlation coefficient of 0.94 and with a resultant equation of Y (mg water/mg dry SC) = 0.00027X (conductance) ± 0.45435.

Effect of Multiple Stratum Corneum Sheets The effect of increasing the number of SC sheets on recorded conductance values was studied in a room with a RH of 45% and a temperature of 26°C. It is noteworthy that the water content measured with each model,

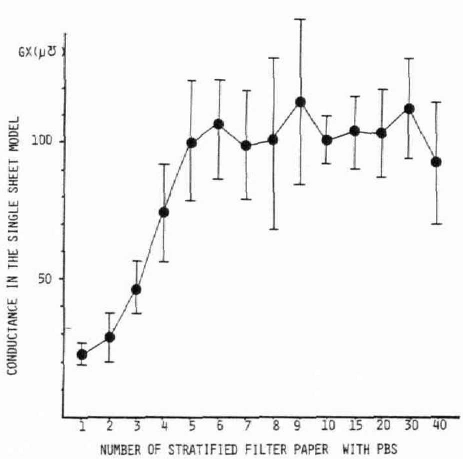

Figure 3. The change of conductance as a function of thickness of the conducting medium under a sheet of stratum corneum measured at 45% RH and 26°C.

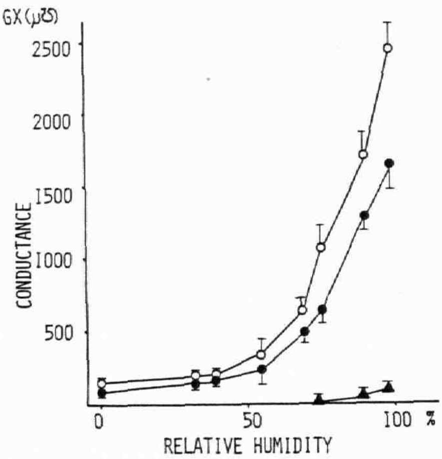

Figure 5. Relationship between conductance and relative humidity. Excised whole skin piece *(open circles)*; SC in the single sheet model *(closed circles)*; and isolated single unmounted SC sheet *(solid triangles)*.

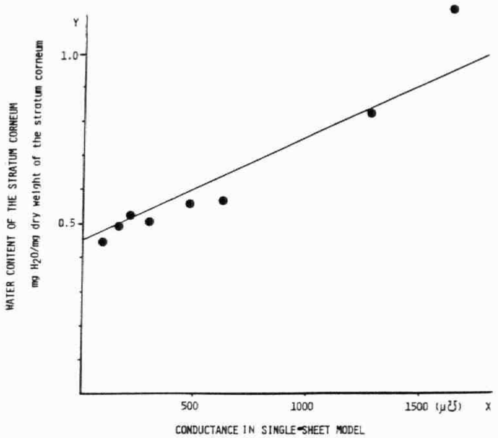

Figure 6. Correlation between the conductance recorded for the single sheet model *(X)* and the water content of the SC of the model *(Y)*. Y = 0.00027 X + 0.45435 (r = 0.94).

i.e., the single-, three-, and five-sheet models, was almost the same despite the difference in thickness of total SC, i.e., 0.543 ± 0.102, 0.523 ± 0.080, and 0.545 ± 0.0067 mg water/mg dry SC, respectively. As shown in Figure 7, however, conductance values measured on the surface of the uppermost sheet decreased inversely with the number of SC sheets.

Figure 8 illustrates profiles of the water concentration gradient in each sheet of the five-sheet model. The sheet numbers in this figure corresponds to those of the five-sheet SC model shown in Figure 1. The water concentration progressively increased, following almost straight parallel lines, from the outermost sheet (No.1), about 0.1 mg water/mg dry SC to the innermost sheet (No. 5), more than 0.8 mg water/mg dry SC, at RH between 0 and 75% RH. At higher RH the water content of every SC sheet increased measurably, particularly in those closest to the outside.

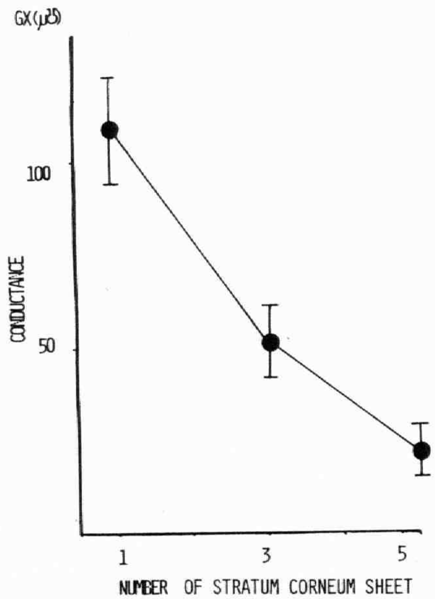

Figure 7. The relation between conductance and number of SC sheets mounted on the model under the indoor condition of 45% RH and 26°C.

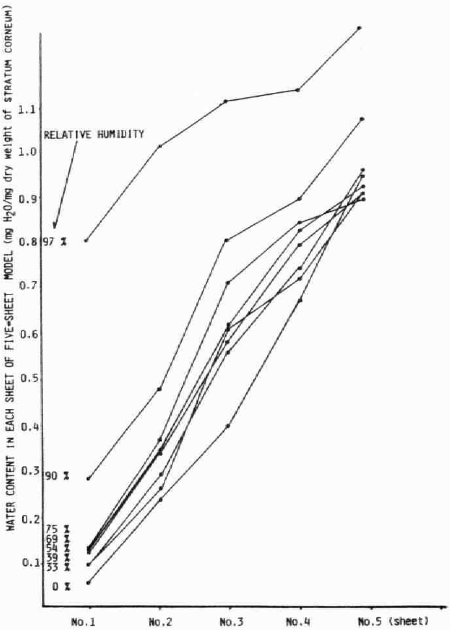

Figure 8. Water content measured for overlying sheets of SC in the five-sheet model placed in environment with RH ranging from 0% to 97%. The SC sheets were numbered from the outermost (No. 1) to innermost (No. 5). Bars for standard errors were omitted from the graphs.

Figure 9 summarizes the relation between the water content in the five-sheet model of SC and the environmental RH. The total water content of the five-sheet model showed a curve almost similar to that obtained for the single sheet model as shown in Figure 4, despite the fivefold increase in thickness of SC. The curve of water content in sheet No. 1 resembled that of the isolated uniformly hydrated SC sheet (Fig 4), although it showed slightly higher values at every level of RH than the latter.

The curve obtained from conductance values plotted in the five-sheet model at various RH (Fig 10) also resembled that of the single-sheet model (Fig 5). Interestingly, the conductance values mea-

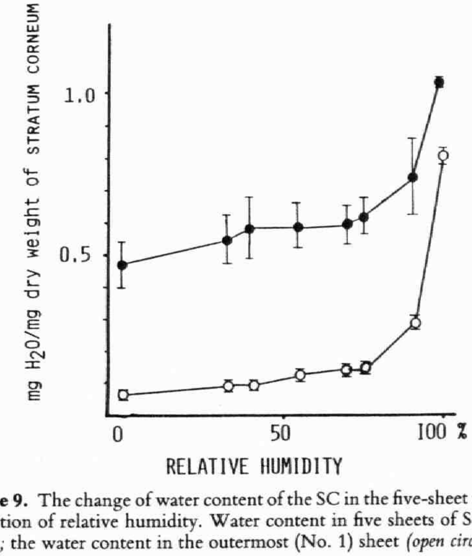

Figure 9. The change of water content of the SC in the five-sheet model as a function of relative humidity. Water content in five sheets of SC *(closed circles)*; the water content in the outermost (No. 1) sheet *(open circles)*.

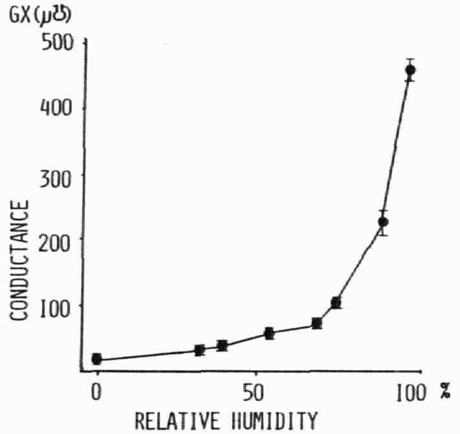

Figure 10. The change of conductance in the five-sheet model as a function of environmental RH.

sured at every RH were in a range of one-fifth of those noted for the single-sheet model.

When the water content in sheet No. 1, the outermost sheet, or the total water content of the five SC sheets were compared with the conductance values obtained in the five-sheet model, the water content of both had a correlation coefficient of 0.98 and with resultant equations shown in the legend for Figure 11.

DISCUSSION

In a simplified fashion, SC can be depicted as a thin water-proof membrane tightly wrapping fully water-saturated living tissue of the skin. From such an assumption we have developed a simple and convenient SC model. Despite its simplicity, we have obtained evidence indicating that its water-barrier function closely simulates that of the SC in vivo; the magnitude of water evaporation from the single-sheet model is slightly lower than that recorded in vivo, because the evaporation occurs only through a 6-mm-round hole. Hence, we can assume that in this model the passive diffusion of water from the underlying water source takes place only through the SC as in vivo. Use of occlusive vinyl tape for sealing over a wide portion of the SC surface is common practice, and this may increase the hydration of the underlying SC. Gravimetrical determination in

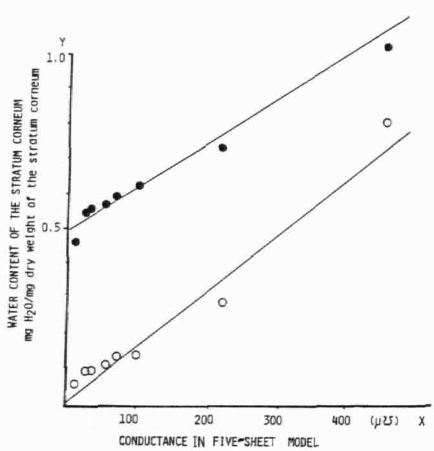

Figure 11. Correlation between the conductance measured on the five-sheet model *(X)* and the total water content of all 5 sheets *(Y)*; Y = 0.00120 X + 0.49356 (r = 0.98) *(closed circles)*. Correlation between the conductance of the five-sheets model and the water content of the No. 1 sheet *(Y)*; Y = 0.00157 X + 0.02814 (r = 0.98) *(open circles)*.

this simulation model, however, as shown in Figure 8, demonstrated that the water content in the superficial portion of SC was still far lower than that of the deepest portion, suggesting that the influence of the occlusive vinyl tape may not be as large as predicted.

Technical assessment has confirmed the accuracy and reproducibility of the conductance measurements using a high frequency current of 3.5 MHz for the in vivo evaluation of the hydration state of the skin surface [16,21]. A relationship between the recorded conductance value and the actual water content in the SC has not been clearly established, however, because we have lacked an appropriate in vitro model of SC in which there exists a concentration gradient of water within the SC as noted in vivo. Using the present simulation model we have substantiated that there is a high correlation between the water content in the SC and conductance with a correlation coefficient of 0.94. Moreover, by changing the thickness of the water-saturated paper pad underlying the sheet of SC, we have noted that a high frequency current of 3.5 MHz must propagate at least 5 mm into the skin to attain an optimal reading, much deeper than previously expected. In the model, probably the wet paper can be replaced by any other kind of wet electrically conductive substances, because in vivo the recorded conductance values are not influenced to any degree by the components of underlying tissue, whether or not they are healthy skin components or just blister fluid, as long as the overlying SC is intact [4].

As in in vivo, the exposed surface of the SC in this model remained soft and flexible without developing cracking, even in a very dry environment. Our gravimetrical determination demonstrated that such SC contained about 45 mg water/mg dry SC. These findings are in distinct contrast with those reported for an isolated piece of plantar SC, which only became soft and pliable when a moisture content of about 10 mg/100 mg dry weight was reached at a RH of 60% [19]. According to our previous estimation, dry normal SC has the capacity to bind water molecularly within a limit of 33 mg/mg dry SC; water exists in SC in a liquid state as free water above this limit [21]. Therefore, the present results suggest that normal SC contains at least some free water fraction even at an RH of 0%. The free water is thought to exist mainly in the lower portion of the SC close to the water-saturated living tissue, because the gravimetric determination in the five-sheet model demonstrated that the lowermost part of the SC contained water equivalent to about 90% of its dry weight, whereas the uppermost SC sheet contained only 5% water (Fig 8).

In a strict sense, the five-sheet model differs from a single sheet of thick SC because the former consists of five repeating functionally different portions of SC in contrast with the latter in which a functional change occurs from the lowermost to the uppermost portions gradually. We observed, however, that the total water content of the SC was not different between the single-sheet model and the five-sheet model, not influenced by the thickness of the SC. Thus, the five-sheet model of SC can be simply regarded to be a fivefold thick model of normal SC. In fact, we found an inverse relationship between the conductance value and the number of SC sheets, i.e., the thickness of the SC. The hydration state of the superficial portion of the SC depends mainly on the water that diffuses from the underlying wet living tissue, and the amount of water that reaches the surface decreases inversely to the thickness of the SC as calculated from Fick's diffusion equation [5].

In the past we have been unable to measure the water content of the innermost layer of the SC in vivo, but have speculated that it is probably in equilibrium with the adjacent moist living epidermis [5]. In the present study, we determined the water content in the deepest portion of the SC by measuring the water content of the lowermost SC sheet of the five-sheet model. The water content thus determined was equivalent to about 89 mg/mg dry weight of SC. This value is close to 88%, which was estimated by Blank et al [5] from the water content of the living soft tissue or to that of the dermis soaked in saline [22]. However, it was considerably higher than the value of 40% recently calculated by Warner et al [23], who assumed that the water content of the viable epidermis was about 70%. Such higher values for water content in our SC model are chiefly due to our assumption that the SC directly covers fully hydrated living tissue, i.e., we have ignored the possibility of the presence of a water barrier in the uppermost portion of the epidermis. According to the recent report of Warner et al [23], there was a profound decrease in water content of the epidermis at the last stratum granulosum layer, probably due to the presence of the lipid-enriched intercellular domain produced by granular cell secretion of lamellar bodies [24].

There has also been much speculation as to whether the water concentration profile of the SC in vivo approaches a straight line or whether the first two- or three-cell layers are quite dry and the remaining cell layers relatively wet. Recently, Blank et al [5] calculated theoretically that it follows a straight line. Warner et al [23] observed by electron probe analysis of rapidly frozen human epidermis that this profile is approximately linear. Our present data from the five-sheet model also clearly demonstrates an almost straight water concentration gradient within the SC gravimetrically (Fig 8).

In contrast with the deeper portion of the SC that contains both bound and free water, it seems likely that the upper part of the SC holds water only in the form of bound water under the ambient conditions of RH ranging from 0 to 75%; even at 75% RH, the uppermost SC sheet was found to contain water equivalent to about 15% of its dry weight. This value compares well with that of Warner et al [23], although they did not specify at which RH they obtained their skin samples. Only at a very high RH of 97%, where transepidermal water loss might be nearly zero [5], did a large amount of water begin to accumulate in the superficial portion of the SC as free water.

The five-sheet model has also clearly shown that the water content in each level in the SC is relatively uninfluenced by the change of environmental RH (Fig 8). It is remarkable that there was only a slight change in the water profile of SC over a wide range of environmental RH between 0 and 75%. In extremely humid conditions with RH higher than 90%, however, the water content increased greatly even in the lowermost layer of the SC; the water content in the lowermost layer of the SC seems to exceed that of the reported underlying epidermal tissue [5,23]. Thus, presumably prolonged exposure of the skin to an excessively humid environment, e.g., under an occlusive diaper, in the hands or feet immersed in water, or in the intertriginous areas with profuse sweating, may induce a deleterious effect not only on the SC, but also on the living tissue, with a resultant dermatitic change with maceration.

In this five-sheet model we also found a good correlation between the conductance and the water content in the outermost portion of the SC as well as that in the whole SC (Fig 11). However, Figure 11 shows two lines that somewhat converge, seemingly induced by the data obtained at extremely high humidity, because the superficial portion of the SC tends to be greatly affected by the abnormally high humidity of the environment. If these high humidity data were omitted because of the abnormal conditions, then the lines appear to be parallel, suggesting that the water content of the skin surface closely reflects that in the whole SC and also substantiating the predictive accuracy of the conductance measurements under usual ambient conditions.

In conclusion the present in vitro analyses using a model simulating in vivo SC provides consistent and convincing evidence that the high frequency method accurately assesses the hydration state of the SC. In addition the simulation model of in vivo SC is a useful substitute for human volunteers, particularly for the functional study of SC under extreme environmental conditions where in vivo experiments are not feasible.

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